Assessment and reproducibility of mitochondrial function and mitophagy measurements in human muscle tissue of active and pre frail elderly subjects

Published: 15-05-2015
Last updated: 19-04-2024

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Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Other condition
Study type	Observational invasive

Summary

ID

NL-OMON42565

Source
ToetsingOnline

Brief title
Mitophagy in elderly subjects

Condition

- Other condition
- Musculoskeletal and connective tissue disorders NEC

Synonym
defect of the powerhouse of the cell, Mitochondrial dysfunction

Health condition

mitochondriele dysfunctie

Research involving
Human

Sponsors and support

Primary sponsor: Amazentis SA
Source(s) of monetary or material Support: Bedrijf: Amazentis SA

Intervention

Keyword: Mitochondrial dysfunction, Mitophagy, Sarcopenia

Outcome measures

Primary outcome

To assess the level of mitophagy and autophagy in muscle biopsy tissue of active healthy elderly subjects and sedentary pre-frail elderly subjects using 1) gene expression and 2) protein expression analysis for candidate genes linked to mitophagy and autophagy biological process.

Secondary outcome

- PCr recovery time (in seconds) measured by 31P-MRS.

- Ratio of mtDNA to nuDNA measured by qPCR.

- Activity of citrate synthase and respiratory complexes measured by assay in muscle tissue.

- mVO2 (in ml/min/100 ml) in muscle measured by NIRS.

- MitoPO2 (in mmHg) in the skin measured by PpIX-TSLT.

- Hand grip strength (in kg) measured by the Jamar dynamometer.

- Strength during maximum voluntary isometric contraction of the quadriceps (in kg).

- Postural stability (in cm sway) measured by body sway.

- Ability to maintain standing balance (yes/no), as part of the Short Physical

Performance Battery (SPPB).

- Level of activity, using the Vital Connect Healthpatch accelerometer.

- 4-meter walking speed (in m/s), as part of the SPPB.

- Sit-to-stand transfer (in s), as part of the SPPB.

Study description

Background summary

Age related diseases pose a burden for both the elderly and society as a whole. In recent years, evidence has shown that dysfunction of mitochondria plays an important role in age related diseases, such as Alzheimer*s and Parkinson*s diseases, diabetes mellitus type 2 and sarcopenia. The mitochondrion is a central organelle that can drive both cellular life, i.e. by producing energy in the respiratory chain, and death, i.e. by initiating apoptosis. More recently, it was demonstrated that dysfunctional mitochondria can be specifically targeted for elimination by autophagy, a process that has been termed mitophagy. During aging, there is a progressive decline in the cell capacity to eliminate its dysfunctional elements by autophagy, as evidenced by the accumulation of oxidative damage and mutations in mitochondria and the decrease in autophagic flux. Therefore, restoring levels of autophagy and mitophagy in the elderly represents an interesting therapeutic approach to improve mitochondrial function. Most of the compounds that have been identified to improve mitochondrial function either stimulate mitochondrial biogenesis (e.g. AICAR, resveratrol, nicotinamide riboside) or the respiratory chain (e.g. coenzyme Q10), while no specific mitophagy inducer has been identified yet. A major challenge posed lies in the lack of tools to quantify mitophagy and the necessity to have accurate measurements to observe an improvement in mitochondrial function in vivo in humans. The discovery of the dynamic process of mitophagy represents an important finding towards an effective therapeutic strategy for aging-related mitochondrial-based conditions. However, since this is a relatively recent discovery, only a few markers specific mitophagy markers have been identified to date. These include parkin, an E3 ubiquitin ligase, and Phosphatase and tensin homolog (PTEN)-induced putative kinase protein 1 (PINK1), two genes in which mutations have been linked to hereditary forms of Parkinson*s diseases. Under basal conditions, PINK1 is imported into mitochondria and rapidly turned over by proteolysis. When mitochondria are dysfunctional, PINK1 accumulates at the surface of the organelle and recruits Parkin, which can then ubiquitylates mitochondrial proteins. Ubiquitylation is a universal signal for degradation that is recognized by the autophagy adaptors, such as sequestosome 1 (SQSTM1/p62), which can then bind to

microtubule-associated protein 1 light chain 3 beta (LC3B), a protein linked to the autophagosome. The mitochondrion tagged with ubiquitin is then engulfed into the autophagosome to be degraded by lysosomal enyzmes. Most of the work related to these biomarkers was performed in neuronal cell lines, because of its particular relevance for Parkinson*s disease. Therefore, the two most characterized markers of mitophagy (PINK1 and parkin) are mostly applicable to neurons. In muscle cells, it has not yet been established what mitophagy adapters are. In this study, a set of mitophagy biomarkers will be measured in muscle samples from elderly male and female active and pre-frail subjects, in order to validate these biomarkers for further research.
Dynamic 31P-MRS is an established method to measure mitochondrial function and can be considered to be the gold standard for in vivo mitochondrial function measurement. The correlation between mitophagy and mitochondrial dysfunction in neurology is fairly established. However, less is known regarding muscle tissue. Therefore in this study we compare mitochondrial function to level of mitophagy in muscle tissue.

Study objective

Amazentis (AMZ) has identified the first ingredient that is able to stimulate mitophagy, namely AZ400, a natural product derived from food. AMZ has conducted several preclinical studies showing that AZ400 has a strong potential to improve muscle function in adult mice through the enhancement of mitochondrial function via mitophagy. The objective for this study is method and diagnostic assay development for the measurement of mitochondrial function and mitophagy in human muscle tissue of healthy, active elderly subjects and pre-frail, sedentary elderly subjects . The main objective is to assess the level of mitophagy in muscle biopsy tissue of active healthy elderly subjects and sedentary pre-frail elderly subjects using three different methods, including (1) histopathology and staining for microtubule-associated protein autophagy marker Light Chain 3 (LC3), (2) quantification of ubiquitylation of the mitochondrial fraction, and (3) gene expression.

Study design

Method validation study of biomarkers, parallel group design, with two consecutive study periods for assessment of day-to-day reproducibility of measurements.

Study burden and risks

A needle muscle biopsy is a safe and well tolerated method to obtain muscle tissue. In this study, the muscle biopsy procedure will be performed twice to determine the reproducibility of mitophagy biomarkers. In a study in elderly subjects, 87% of subjects indicated that they would undergo a muscle biopsy again. The type of muscle biopsy in this study was more invasive and more

burdensome than the method proposed in the current study. Knowledge about the variability is required to determine the validity of the assessments and to assess the sample size for the phase 1 clinical trial involving AZ400. Therefore, in our opinion, the benefit of repeating the procedure outweighs the burden for the subjects. The other techniques to measure mitochondrial function, including MRI scanning, performed in this study are non-invasive procedures, that yield only little burden for the subjects.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

Inclusion criteria for active, healthy subjects;1. 61 years of age or older, inclusive.
2. Healthy male or female subjects. Healthy status is defined by absence of evidence of any active or chronic disease following a detailed medical and surgical history, a complete

physical examination including vital signs, 12-lead ECG, haematology, blood chemistry, and urinalysis.
3. Body mass index (BMI) between 15 and 35 kg/m2, inclusive.
4. Able to participate and willing to give written informed consent and to comply with the study restrictions.
5. Category 2 or 3 as assessed by the International Physical Activity Questionnaires (IPAQ). Activity level is >= 600 MET (metabolic equivalent unit) - minutes per week.
6. Normal physical performance: normal gait speed, i.e. a walking >= 0.8 m/s in the 4-m walking test.
7. Normal muscle mass: normal skeletal muscle mass index (SMI), measured by Bioimpedance analysis (BIA, for males >= 10.75 kg/m2, for females >= 6.75 kg/m2).
8. Normal muscle strength: handgrip strength (measured with the Jamar dynamometer) of >= 30 kg for males and >= 20 kg for females.;Inclusion criteria for sedentary, pre-frail subjects;1. Sedentary, pre-frail males. Pre-frailty is defined as fulfilling to at least two out of the following three criteria: low physical performance (low gait speed, i.e. a walking speed below 0.8 m/s in the 4-m walking test), low muscle mass (a low skeletal muscle mass index (SMI), measured by Bioimpedance analysis (BIA, for males < 10.75 kg/m2, for females < 6.75 kg/m2)) and/or low muscle strength: handgrip strength (measured with the Jamar dynamometer) < 30 kg for males and < 20 kg for females. Sedentary behaviour is defined as having an activity category of 1 as assessed by the International Physical Activity Questionnaires (IPAQ) (Activity level is < 600 MET (metabolic equivalent unit) - minutes per week).
2. Body mass index (BMI) between 15 and 35 kg/m2, inclusive.
3. Able to participate and willing to give written informed consent and to comply with the study restrictions.
4. 61 years of age or older, inclusive.

Exclusion criteria

Exclusion criteria for active, healthy subjects;1. Presence of any contraindication to have MRI scans performed (e.g. pacemaker, intracranial clips etc.).
2. Having diabetes mellitus or lower extremity peripheral vascular disease, as these conditions may interfere with interpretation of the dynamic 31P-MRS and NIRS of the lower extremity.
3. Participation in a clinical trial within 90 days of screening or more than 4 times in the previous year.
4. A history (within 3 months of screening) of alcohol consumption exceeding 3 standard drinks per day on average (1 standard drink = 10 grams of alcohol).
5. Inability to refrain from smoking more than half a pack of cigarettes (or similar for other tobacco products) per day during the course of the study (from screening to End-of-Study [EOS]).
6. A history or presence of allergy to 5-aminolevulinic acid or porphyrins.
7. A history or presence of allergy to lidocaine.
8. Positive hepatitis B surface antigen (HBsAg), hepatitis C antibody (HCV Ab), or human immunodeficiency virus antibody (HIV Ab) at screening.

9. Loss or donation of blood over 500 mL within three months (males) or four months (females) prior to screening.
10. Unwillingness or inability to refrain from consuming alcohol within 48 hours before each visit until the end of that visit.
11. Unwillingness or inability to refrain from consuming 8 or more units of xanthine containing beverages and foods per day during the entire study.
12. Unwillingness or inability to refrain from consuming the following supplements: L-carnitine, creatine, Q10, vitamin A, niacin, folic acid, vitamin C, vitamin E and probiotic- foods and supplements at least two weeks before study enrolment.
13. Unwillingness or inability to have a muscle biopsy performed.;Exclusion criteria for sedentary, pre-frail subjects;1. Presence of any contraindication to have MRI scans performed (e.g. pacemaker, intracranial clips etc.).
2. Having diabetes mellitus or lower extremity peripheral vascular disease, as these conditions may interfere with interpretation of the dynamic 31P-MRS and NIRS of the lower extremity.
3. Participation in a clinical trial within 90 days of screening or more than 4 times in the previous year.
4. A history (within 3 months of screening) of alcohol consumption exceeding 3 standard drinks per day on average (1 standard drink = 10 grams of alcohol).
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12. Unwillingness or inability to refrain from consuming the following supplements: L-carnitine, creatine, Q10, vitamin A, niacin, folic acid, vitamin C, vitamin E and probiotic- foods and supplements at least two weeks before study enrolment.
13. Unwillingness or inability to have a muscle biopsy performed.
14. Underlying chronic disease, which, in the opinion of the investigator would interfere with study participation or the validity of the measurements.
15. Unintentional weight loss <=5% of usual body weight during the last 6 months.
16. Anorexia or anorexia-related symptoms

Study design

Design

Study type: Observational invasive

Masking:	Open (masking not used)
Control:	Uncontrolled
Primary purpose:	Diagnostic

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	15-06-2015
Enrollment:	20
Type:	Actual

Ethics review

Approved WMO Date:	15-05-2015
Application type:	First submission
Review commission:	BEBO: Stichting Beoordeling Ethiek Bio-Medisch Onderzoek (Assen)
Approved WMO Date:	07-12-2015
Application type:	Amendment
Review commission:	BEBO: Stichting Beoordeling Ethiek Bio-Medisch Onderzoek (Assen)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL53006.056.15